Chapter 6 General discussion and conclusions

Typhoid fever poses an important threat to public health in many developing countries and this peril is especially pronounced in the Southeast Asian region with its rapid pace of economic development. Papua New Guinea and Indonesia have the highest annual incidences of typhoid fever with nearly 1000 cases per 100 000 population (Pang et al., 1995), but there is an alarming increase as well in multidrug- resistant *S. typhi* strains in the Indian subcontinent and Vietnam (Shanahan et al., 1998; Wain et al., 1999; Connerton et al., 2000; Shanahan et al., 2000)

Countries that report increased typhoid severity share characteristics that result from many interrelated factors including increased urbanization and population, overburdened health care services, increase of antibiotic resistance, variable efficacy of vaccine preparations, inadequate supply of clean water and increased regional movement of migrant workers (Edelman and Levine, 1996).

The continuing presence and burden of this disease in endemic areas where periodic outbreaks happen frequently and its potential for spread mean that improved epidemiological surveillance is needed. Documentation of the extent of genetic variations among *S. typhi* strains is also of utmost importance. This has led to the development of phenotypically and genotypically based typing methods. These techniques for analyzing microbial relatedness have become critical (i) for identifying outbreaks of infection, (ii) for determining the mode and source of acquisition of a pathogen, (iii) for recognizing particularly virulent strains of organisms, (iv) for analyzing individual patients to determine if a series of isolates obtained over time represents relapse of infection due to a single strain

or separate episodes of disease from different infections, and (v) for defining effective preventive (vaccination programs) and therapeutic measures (Olive and Bean, 1999).

Typing methods for strain differentiation must meet several criteria in order to be broadly useful. The important criteria includes (i) typeability, refering to the ability to produce an interpretable positive result, (ii) reproducibility, refering to the ability of a technique to produce the same result when the same strain is tested repeatedly, and (iii) discriminatory power refering, to the ability to differentiate among unrelated strains. The other important criterias include ease of performance, ease of interpretation of data/results, cost, and rapidity of the method (Olive and Bean, 1999).

Our adaptation of the use of agarose plugs in the DNA extraction process, which is a commonly used method for pulsed-field gel electrophoresis, has provided a convenient, safe and reproducible method for preparing bacterial DNA for ribotyping, IS200 typing and gene hybridization studies using endonuclease- restricted DNA. Standardization of DNA extraction is an important criteria in molecular typing. Waterhouse and Glover (1993) noticed that hybridization patterns of *Bacillus subtilis* genes coding for rRNA varied according to the method of DNA preparation utilized. The importance of this DNA extraction has been stressed in Chapter 2, especially for certain species such as *Burkholderia* and *Pseudomonas* where problems are usually encountered at this stage due to the abundance of polysaccharide found in the capsules of these organisms.

Phage typing has been the most common method used to date for the demonstration of epidemiological associations among *S. typhi* isolates. However, this typing method has certain limitations such as being technically demanding and can only be performed in reference laboratories (Hickman-Brenner and Farmer, 1983). In addition a significant proportion of strains are not typeable, and some are Vi negative (Cheong et al., 1992).

Based on biochemical studies of enzyme polymorphisms using multilocus enzyme electrophoresis (MLEE), *S. typhi* has been assumed to be a homogeneous and highly clonal pathogen that has shown minimal intraspecies divergence in its spread to different parts of the world. Only one or two clones appear to exist worldwide (Reeves et al., 1989; Selander et al., 1990). This homogeneity among *S. typhi* strains was also observed in the analysis of envelope protein profiles (Franco et al., 1992).

More recently, the development of molecular genotypic typing methods such as PFGE and ribotyping have shown that the extent of genetic diversity among *S. typhi* strains from different regions of the world is actually greater than previously thought (Altwegg et al., 1989; Nair et al., 1994; Thong et al., 1995). Results of our current work clearly indicates the multiclonal nature of *S. typhi* strains from different geographic locales, not only by PFGE and ribotyping, but also by other techniques such as amplified fragment length polymorphism analysis and IS200 typing. These findings are in agreement with the recent observation that microbial populations have shown substantial genetic diversity within species, pointing to the fact of isolates being distributed among many genetically diverging lineages (Arbeit, 1995). This evolutionary divergence might be due to the accumulation of random, nonlethal point mutations including single base substitutions and deletions, deletion of single genes or even substitutions of DNA from other microbial species (Arbeit, 1995).

There is evidence from field studies and epidemiological data based on molecular techniques that in different parts of the world the disease manifestation of typhoid fever can vary in severity (Hoffman, 1991). One of the factors for this varied manifestation could be due to isolates that are genetically different, hence possessing different degrees of virulence. It has been suggested as well that the variable efficacy of typhoid vaccines such

as the oral Ty21a and parenteral Vi polysaccharide in various geographical regions might be the result of genetic variations in strains present in these countries (Pang, 1998b). Therefore, efficient subtyping techniques are important for effective surveillance of the multiclonal *S. typhi* strains and in identifying the presence of virulent isolates.

An important finding of the present study was the lack of genetic diversity observed among the *S. typhi* strains from Papua New Guinea, even though they were isolated over a period of 3 years. The limited diversity observed is most probably related to the fact that typhoid fever was only relatively recently introduced into PNG (rarely seen prior to 1985) (Passey et al., 1985). Presumably, the *S. typhi* strains circulating in PNG were derived from a single clone or have the same genetic lineage. These strains have now spread with the development of some limited genetic variation. These limited variations are probably due to the minimal selective pressure encountered by the endemic *S. typhi* strains. There was a temporal pattern to the changes as the PFGE and ribotype profiles for the PNG strains slowly changed from 1992 to 1994. It is noted in this current study that different typing methods assess different segments of the chromosome which evolves differently through space and time. The PFGE approach is a measurement of diversity throughout the entire genome which includes mutations at restriction enzyme (RE) cut sites, addition or deletion of mobile genetic elements and insertion, deletion, duplication or inversion of DNA segments (Maslow et al., 1993). In contrast, IS200 analysis and ribotyping only reflect variability in certain parts of the genome. IS200 profiles depend on the RFLPs at the insertion sites and from the DNA rearrangements following the transposition of the IS element into the genome (Stanley et al., 1993). Ribotypes on the other hand are dependent on genetic rearrangements between *rrn* operons and polymorphisms at RE cut sites of the enzymes used (Ng et al., 1999). Like PFGE, AFLP analysis provides a way of examining

DNA segments over the entire genome of an organism. AFLP is able to detect mutations at RE cut sites of the frequently cutting enzyme used and mutations in the sequence adjacent to the RE cut sites which are complementary to the selective primer extensions used (Savelkoul et al., 1999). These AFLP genetic markers change more rapidly in evolution than the markers detected by PFGE analysis, IS200 fingerprinting and ribotyping. This may explain why the discriminatory index for strain differentiation achieved by AFLP is higher than that of PFGE, ribotyping and IS200 typing. On the other hand, the PFGE markers evolves faster than the IS200 and ribotyping markers and thus shows the higher discrimination level of PFGE compared to IS200 typing and ribotyping. IS200 markers evolve very slowly and is a good way of showing the clonality of the PNG strains, as only one IS200 profile was observed for all the PNG strains assessed. From the analysis of the PNG isolates, we conclude that the genetic diversity is a function of the typing method used and a function of time.

A key question follows : Why do the results of this present study which exposes the significant genetic diversity among *S. typhi* isolates go against earlier findings of limited clonality assessed by MLEE? As has been mentioned, molecular genotyping methods detect genetic diversity more efficiently due to the rapid evolution of the genetic markers targeted, as compared to the slower rate of evolution of the housekeeping enzyme markers detected by MLEE. This explains the clonality observed by MLEE analysis (Selander et al., 1991). It must be stated that the definition of clonality should used as a relative concept rather than an absolute condition, as it is dependent on the typing method used (Stanley and Saunders, 1996). MLEE data are useful in evolutionary studies for the detection of long term genetic variations, while data from DNA based techniques can monitor short term variation (Pang, 1998b). Young (1989) expressed that the population genetics of

pathogenic bacteria is characterized by "high genetic diversity and a strongly clonal population structure". A similar view has also been pointed out by Pang (1998b) in which he mentions that the existence of diversity within clonality for *S. typhi* should be considered. The results of this present study clearly strengthens this notion. PNG *S. typhi* strains derived from a clonal population is slowly diverging to form clonal variations. The clonal variations are derived due to selective pressures the strains encounter in their environment.

What needs to be stressed here is that there is no "gold" standard method for typing *S. typhi*. The choice of a suitable typing technique strictly depends on the epidemiological settings. This has been well documented. For example; PFGE, IS200 typing and RAPD-PCR was able to discriminate multidrug resistant *S. typhi* isolates from sensitive strains (Threlfall et al., 1993; Shanahan et al., 1998). Analysis of *S. typhi* from sequential blood samples by PFGE, plasmid profiling and ribotyping was able to distinguish relapse from reinfection cases in individuals suffering form typhoid fever (Wain et al., 1999). Ribotyping and PFGE analysis have shown diversity among *S. typhi* isolates associated with sporadic and outbreak cases (Altwegg et al., 1989; Thong et al., 1994; Echeita and Usera, 1998). Environmental isolates have also been characterized by PFGE (Thong et al., 1996c). Threlfall (1998) emphasized that DNA based typing methods such as PFGE, IS200 typing, ribotyping, PCR-based methods, plasmid profiling and plasmid fingerprinting should be used in complementation with each other and in conjunction with standard established methods such as serotyping and phage typing to solve epidemiological problems related to *Salmonella*.

As previously pointed out, the differences in geographical variations of disease severity are unclear, but may involve factors such as differing levels of endemicity,

differences in health care facilities, host immune response, genetic factors and as has been mentioned earlier, perhaps strain variation among the causative agent. Up until now limited studies have been performed to determine if variations in clinical presentations are related to strain differences. Franco et al. (1992) and Grossman et al. (1995) showed that there was little correlation between strain characteristics and disease severity. Previous studies by Thong et al. (1996b) have shown a correlation between molecular profile of *S. typhi* as defined by PFGE and its ability to cause fatal disease among typhoid fever cases in Papua New Guinea. They conclude the possibility of association between genotype, as assessed by PFGE, and the capability to cause fatal illness. However analysis of *S. typhi* strains isolated from patients with fatal/non-fatal typhoid fever in Papua New Guinea by AFLP and PFGE in this present study suggested no such correlation between genotype and virulence. Based on our findings, we assume that no particular *S. typhi* genotype assessed by either PFGE or AFLP analysis seems to be particularly more virulent, as the ability to cause fatal disease is not associated with the banding patterns produced by either technique. Therefore, other factors (including host factors), which are as yet unknown, may be involved in the causation of fatal disease.

Using a proper typing method can be advantageous in detecting *in vivo* genetic changes of strains isolated from various body parts of a patient during the course of infection. AFLP was able to detect genetic changes in the PNG *S. typhi* strains isolated from blood and feces of the same patient. PFGE and ribotyping were less sensitive in detecting these changes. These observations underline the importance of the typing technique used as each method assesses different genetic markers which evolves differently through space and time. Such analysis may help to define the pathogenesis of the infection and the role of other concomitant factors such as exposure of pathogenic strains to host

defenses and antimicrobial agents. It is also of value in assessing the *in vivo* genetic stability of a particular bacterial genome. This will enable the monitoring of strains to see whether resistance develops especially after antibiotic use. Assessment of genetic instability/stability also provides insight to whether a patient is being infected simultaneously by more than one strain of the organism. This was seen in one of the PNG patients, where there is a likelihood that this particular patient was infected simultaneously by two closely related *S. typhi* strains, due to the different AFLP, PFGE and ribotype profiles observed. Results of such a study might be important in patient management and treatment.

Hybridization studies of restricted *S. typhi* DNA with various gene probes related to virulence produced data that clearly indicated the clonal nature of the Papua New Guinea *S typhi* strains and the multiclonal nature of the geographic *S. typhi* strains. The gene probe typing results supports the PFGE, IS200, ribotyping and AFLP data in delineating the *S. typhi* strains studied. By selecting a suitable array of gene probes (after screening for polymorphisms), this method has the potential to be used in molecular epidemiological and evolutionary studies of *S. typhi*, not only for the differentiation of strains but also to detect clonal lineages of this organism.

Data produced from hybridization analysis indicated the plastic nature of most of the genes studied as they produced polymorphic gene probe profiles. This indicates that these genes are located in different regions of the genome. We used PCR-SSCP in the hope of detecting base pair mutations within these translocated genes. SSCP is more convenient to use than sequencing for the detection of base pair polymorphisms in epidemiological studies. PCR-SSCP analysis as a typing tool for the Papua New Guinea and geographic *S. typhi* isolates using various genes proved to be poorly discriminatory as no polymorphisms

comparative epidemiology, it is necessary to develop standardized protocols and also to standardize the interpretation of results. It has been suggested by Struelens et al. (1998), that for the purpose of epidemiological surveillance, monitoring clonal spread and prevalence in populations over extended periods, as would be important in a disease like typhoid fever, will require what is referred to as a "library" typing system. Such methods must be easily standardized, have a high throughput, be quantitative and also adopt a uniform nomenclature (Struelens et al., 1998). The issue of standardization is particularly important as it would enable results from the same laboratory (intralaboratory) and different laboratories (interlaboratories) to be compared. Of the many library typing systems, it has been proposed that genotypic profiles generated by AFLP are particularly suitable, especially if they are more reproducibly and objectively analyzed by using electrophoresis with automated laser detection with the aid of computer software (Struelens et al., 1998).

The other typing methods that fit into this "library" typing system are PFGE, ribotyping, IS200 typing and some of the PCR-based typing schemes like PCR-SSCP. Tenovar et al. (1995) have proposed a system for standardizing the interpretation of PFGE patterns in relation to determining strain relatedness. With the aid of computerized gel scanning and analysis software, it is possible to create data banks of PFGE patterns for *S. typhi* strains, enabling the creation of reference databases, to which any new strain could be compared for identifying its phylogenetic relationship to other similar strains (Olive and Bean, 1999).

Ribotyping was originally suggested as a method for taxonomic studies but it is now more commonly used for typing purposes. Comparison of ribotype patterns originating from different laboratories had been hampered in the past due to differences in

ribosomal probes used. Standardization of these *rrn* probes, computer assisted image analysis and statistical treatment of the data will probably speed up and improve the reliability of ribotype pattern comparison (Bingen et al., 1994; Olsen et al., 1997). More extensive databases are becoming available for standardized ribotyping through a commercially available automated system (RiboPrinter, Qualicon, Wilmington, DE).

IS200 typing results can also be displayed in a band-matching database with the aid of computerized scanners and software for strain identification, as suggested by Stanley et al. (1993). This will allow its use in epidemiological studies not only of regional but also of international outbreaks. An example of standardized protocols of IS fingerprinting is seen in the typing of *Mycobacterium tuberculosis*, which integrates standardized computer analysis of patterns and a common database, and is now widely used for large-scale surveillance of tuberculosis (van Embden et al., 1993).

Multiple fluorescence based PCR-SSCP performed with an automated sequencer has provided the capacity for simultaneous labeling of several fragments as well as the potential for an automated data collection system (Jordan et al., 1999).

In the future, the power of genotypic hybridization schemes should be greatly boosted by the use of high-density DNA probe assay, also known as DNA chip technology (Chee et al., 1996). It is basically a non-gel based sequencing method on hybridization of unknown sequences to a DNA array. Detection of fluorescent-labeled target DNA can be performed rapidly by epifluorescene confocal scanning (GeneChip System, Affymetrix, Santa Clara, CA) (Struelens et al., 1998). If this "library" typing scheme could be achieved, then the dream of forming an effective global epidemiological surveillance system is within reach.

Molecular typing techniques not only can be used for epidemiological surveillance of organisms, but it can also be used to dissect the genome of microorganisms to detect genomic reorganizations which lead to genetic diversity. The application of a variety of techniques and approaches including PFGE, ribotyping, AFLP, IS200 typing and gene probe typing has already disclosed the extent of genetic diversity among the geographic *S. typhi* isolates and the clonality among Papua New Guinea isolates studied. This observation on genetic diversity suggest that the *S. typhi* genome is capable of rapid changes and variations. *S. typhi* seems to be more susceptible than other Enterobacteriaceae to genetic reorganization that do not substantially alter the stability and survival of the bacterium (Liu and Sanderson, 1995). This genomic rearrangement (or fluidity of the genome) probably plays a major role in the virulence of *S. typhi* and helps in its ability to survive in the human host (Liu and Sanderson, 1995).

Genetic changes which occur during the evolution of *S. typhi* can be mediated by a number of mechanisms but the resulting changes fall into a few classes:

(a) The *S. typhi* genome has undergone extensive chromosomal rearrangements which mainly involves recombination between *rrn* operons (Liu and Sanderson, 1996). Recombination between other repeat sequences or insertion/deletion of large blocks of DNA also occurs but at a lower rate (Liu and Sanderson, 1996). This chromosomal event suggests that the gene order of *S. typhi* has relaxed during its evolution.

(b) LeClerc et al. (1996) has mentioned the existence of hypermutable phenotypes among *E. coli* and *Salmonela enterica* strains that have mutations at the methyl-directed mismatch repair (MMR) genes. MMR is a post replicative repair system that corrects mismatches on newly synthesized DNA strands. This is to ensure the

precision of chromosome replication. It has been proposed that inactivation of the MMR system will relax normal recombination barriers to allow rapid mutations in the organism's own genes, but also to allow the pathogen to acquire DNA (non-homologous DNA) from distantly related genomes by horizontal gene transfer resulting in pathogenicity islands. Horizontal transfer plays a major factor during evolution of the bacterial chromosome (Cebula and LeClerc, 1997) and, due to this, there is a rapid penetration of virulence genes within the prokaryotic community. Of the known mechanisms by which bacteria diverge, differences in the genome size might be of the greatest significance. Horizontal transfer of novel genes or gene clusters that contribute to pathogenicity are likely causes of these variations (Liu et al., 1999). Previous PFGE analysis has also indicated that there are significant variations in the size of the genomes of *S. typhi* isolates (Thong et al., 1997).

There are 5 known pathogenicity islands in *Salmonella* (SPI-1 to SPI-5) (Blanc-Potard et al., 1999). The phylogenetic distribution of the SPI-1 and SPI-2 islands reveals that their acquisition was critical in the development of *Salmonella* as an intracellular pathogen (Groisman and Ochman, 1996). So it was not surprising that in our present study by using hybridization techniques, we were able to detect the existence of SPI-1 and SPI-2 in all the 25 PNG and 19 geographic *S. typhi* isolates. Another simple hybridization experiment showed the presence of SPI-1 and SPI-2 in both clinical and environmental Chilean strains. No polymorphisms were detected within each pathogenicity islands as RFLP profiles were homogeneous for all the Chilean strains studied. The conserved nature of both these islands are not surprising as any polymorphisms detected within the islands may render the organisms to the non-invasive (Mecsas and Strauss, 1996). From the present study

we conclude that the Chilean *S. typhi* environmental strains isolated from the Mapacho River polluted by sewage were actually of human origin due to the existence of both these pathogenicity islands in the genome of the strains. These findings describe the highly flexible nature of *S. typhi* to survive in different stressful environmental conditions.

(c) Mobile genetic elements (e.g. insertion elements) are thought to play an important role in the plasticity of prokaryote genomes. This class of mobile elements which have sequence repeats are capable of promoting various types of genome rearrangements including deletions, inversion and duplications (Haack et al., 1995). In the genome of highly pathogenic *Leptospira interogans* (Zuerner and Bolin., 1997) and *Shigella flexneri* (Rajakumar et al., 1997) IS elements are associated with virulence genes and play a role in the flexibility of the genome. We have found in this study that *S. typhi* isolates assessed have 22-25 copies of IS200 elements. Gene hybridization data from the present study clearly indicated the polymorphic nature of the *gro*EL gene as it transposed to different segments of the genome. Based on the hybridization results, we managed to co- localize the *gro*EL gene and the IS200 element to the same hybridization fragment. This indicates the possibility of close proximity between the IS200 element and *gro*EL gene in the genome, which in turn gives mobility to the *gro*EL gene to transpose around the genome of *S. typhi*. The *gro*EL gene encodes a heat shock protein (*Gro*EL) which protects prokaryotes from various stressful conditions, both intracellular and extracellular (Zügel and Kaufmann, 1999). Being a human pathogen, *S. typhi* is likely to encounter both these extremes of environmental stresses. What we propose is that the *gro*EL gene transposes around the genome of *S. typhi* due to IS200 elements, in order to alter

gene expression to produce different levels of heat shock proteins, depending on the stress level it encounters in different human hosts. PCR-SSCP work indicates the conserved base sequence of the *gro*EL gene, once again indicating that the production of different levels of heat shock protein is not due to base pair mutations within the genes. This strengthens the case for gene location influencing different levels of the *gro*EL expression. Insertion elements have been widely documented as being involved in the assembly and reassorting of genes in the genome, as well in the activation of gene expression (Mahillon et al., 1999). It also has been documented that stress proteins (e.g.: heat shock proteins) affect virulence regulation in many pathogens such as *Vibrio cholerae* and *Listeria monocytogenes* (Gross, 1993).

The genome of *S. typhi* is in constant flux. While point mutations lead to relatively "slow" evolutionary development, the acquisition and excision of large genomic fragments quickly generate new variants of strains which may facilitate rapid adaptation of bacteria to any new niche. It is a known fact that nearly 20% of the differences in chromosomal DNA sequence in the *Salmonella* chromosome are due to horizontal gene transfers (Lan and Reeves, 1996). Chromosomal rearrangement involving the *rrn* operon also plays an important role in producing genetic instability (Liu and Sanderson, 1995). The various ribotypes generated among *S. typhi* strains assessed in this study indicates this genetic event. Based on our experimental data, it is now also reasonable to assume that the numerous *Salmonella* virulence genes responsible for invasiveness and systemic disease could be rearranged resulting in strain variation to influence disease severity.

Like *S. typhi*, the increased instability of the genome is also noted in *Yersinia* spp. (Najdenski et al., 1995) which might be the reason for increased virulence in these

organisms. It has been suggested that the flexibility of the genome might be the precursor for colonization and infection of the organisms to its host (Matic et al., 1997). *In vivo* and *in vitro* stability/instability of *S. typhi* strains are certainly due to selective precursors such as sanitation levels, high attack rates, the use of antibiotics, vaccination programs, host factors and even subculturing (*in vitro*).

How does *S. typhi* differ genetically from other *Salmonella* spp. and which of these changes may be the basis for the unique phenotypes, such as adaptation to humans and the capacity to cause typhoid fever? At present the answer to this questions is still vague, but significant results have been achieved in previous work and in the present study to indicate that we are headed in the right direction in solving this puzzle. The answer to this question will be the ultimate goal of any scientist in the field of typhoid fever and this aspect of research will certainly be a hot topic in the coming years.

However, it must be stated that the limitation of this study was that the sample size (25 Papua New Guinea *S. typhi* strains and 19 strains from various regions) was too small for meaningful statistical evaluation of the degree of genetic diversity observed among the *S. typhi* strains studied. Some of the results in chapter 3 (e.g. : the correlation between disease outcome and genotype profiles as assessed by AFLP and the delineation of *S. typhi* strains) may not be significant statistically but as an observational study, the conclusions were justified.

Ultimately, important insights for understanding the evolution of *S. typhi* and for the development of rational therapeutics and preventive (vaccine) strategies can be achieved. This is the result of improved epidemiological surveillance due to recent advancement in molecular typing methods, better understanding of genetic diversity as a result of genome flexibility/plasticity and its correlation to biological (phenotypic) traits.

Conclusions

- This is the first study in which various molecular subtyping techniques such as AFLP, PCR- SSCP and gene hybridization were applied in the epidemiologic studies of *S. typhi* to assess the diversity of *S. typhi* genome as a whole, diversity at the base pair level of the genes and diversity based on the transposition of genes around the genome. Based on these techniques, clonality of the PNG *S. typhi* strains and the multiclonal nature of the geographic strains were shown. These methods were used in complementation with previously established methods such as PFGE, ribotyping, IS200 typing, PCR- SSCP and PCR- ribotyping to acertain this diversity. All the methods used in the study were typeable and reproducible, with AFLP being the most discriminitory followed by PFGE, ribotyping, gene hybridization, IS200 typing, PCR-SSCP and PCR- ribotyping.
- The interesting point is that slight molecular diversity was observed within the PNG *S. typhi* strains which were isolated in a 3 year period (1992-1994) as different typing profiles were observed for each of the methods used. This indicates that each typing method assessed different segments of the chromosome which evolves differently through space and time. Thus genetic diversity observed in a particular strain may be a function of the typing method used and also time. This confirms previous suggestions that there is no "gold" typing method for *S. typhi* and each typing method depends on the epidemiological setting.
- Molecular typing techniques are not only useful for epidemiological surveillance of organisms, but it can be used to dissect the genome of microorganisms to detect genomic reorganizations which lead to genetic diversity. In this present work the co-

localization of the IS200 element and the *gro*EL gene on the same *Xba*I hybridization fragment was shown. This indicates that there is a possibility that the *gro*EL gene transposes around the *S. typhi* genome is association with the IS200 element.

- PCR- SSCP (with *gro*EL as the target gene) was useful in the detection of base pair mutations to differentiate different *Salmonella* serovars (interserovar discrimination) as well as strains within a given serovar (intraserovar discrimination).